Pathogenesis and pathobiology of brucellosis in wildlife

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Summary
Natural infections by Brucella spp. have been observed in wild populations. Owing to the similarity of lesions and the course of disease across host and pathogen species, the pathogenesis of brucellosis in wildlife is considered similar to that in domestic animals, which has been studied extensively. Similarities include tropism for reproductive and mammary tissues, trophoblast colonisation by the organism, and similar histopathological findings in organs, especially in the reproductive tract. Differences in the disease course exist and are likely to be attributable to immunological and behavioural differences among species. Further study of the pathogenesis and pathobiology of brucellosis in wildlife is expected to yield unique knowledge with application to disease management in both wild and domestic species.

Keywords

Introduction
Brucellosis at the wildlife–domestic animal–human interface is an excellent example of a ‘spillover–spillback’ zoonotic disease. In many instances, such as that of Brucella abortus, the infection was probably originally transmitted from livestock to wildlife which became a reservoir. The infection now ‘spills back’ from wildlife reservoirs to livestock.

Most wildlife brucellosis studies have consisted of serological surveys, and rigorous pathogenesis studies in wildlife species are lacking. Therefore, information on pathogenesis and pathobiology has been gleaned from case reports, surveys, and the few reported experimental infections. These reports tend to support the widely held concept that the pathogenesis of brucellosis in wild species is similar to that in domestic animals, consisting, in brief, of oral/mucosal entry, migration to and colonisation of local lymph nodes via phagocytes, bacteraemia with dissemination to target sites (reproductive organs and mammary glands) and draining lymph nodes, and trophoblast colonisation during pregnancy, often resulting in abortion.

At the cellular level, following cell invasion the organism is contained in a membrane-bound modified phagosome, the Brucella-containing vacuole (BCV), which interacts with early compartments of the endocytic pathway and subsequently undergoes fusion with the endoplasmic reticulum. Lysosomal fusion is ultimately prevented or limited, thereby providing a protected environment in which brucellae can replicate, called the replicative BCV (49, 66). This concept of similar pathogenesis across bacterial and host species is supported by the similarity of lesions produced by Brucella spp. in a wide range of hosts, including those of B. abortus in cattle and bison (54), B. ovis in domestic and bighorn sheep (36), and marine mammal brucellae in bottlenose dolphins (40) (Fig. 1).

Differences in the course of infection or lesions seen among species are primarily attributable to species-specific variation in immune responses and to behavioural differences. Examples include the following. Retained placentas, commonly seen following abortion or parturition in B. abortus-infected cattle and bison (Bison bison), are not observed in elk (Cervus elaphus). Elk normally remove and consume the placenta as it is being delivered (73). Moose
(Alces alces) appear unable to mount a protective immune response against the organism, usually resulting in mortality from *B. abortus* infection (20). The cattle vaccine strain RB51, while safe and moderately efficacious in cattle and bison, is highly abortogenic in reindeer (*Rangifer tarandus*) (2), and produces no protective immunity in elk (35).

**Brucella abortus**

Brucellosis due to *B. abortus* infection was first suspected in wildlife in 1917 when abortions were noticed in the bison herd in Yellowstone National Park in the United States (41). Agglutination tests for *B. abortus* were positive for two animals that had aborted and negative for one that had not. The organism was first isolated from a bison bull from the National Bison Range in Moiese, Montana, in 1930 (6), followed by two Yellowstone bulls in 1932 (74), elk fetuses in northern Wyoming in 1969 (72) and an aborted Yellowstone bison fetus in 1992 (56).

Bison in Wood Buffalo National Park, and bison and elk in the Greater Yellowstone Area, are the remaining reservoirs of the infection in Canada and the United States, respectively. Serological surveys have demonstrated exposure to *Brucella* spp. attributed to *B. abortus* in numerous wild species (8, 42, 70). Natural infection of *B. abortus* has been confirmed by culture in many artiodactyls, including bison (*Bison bison*), elk (*Cervus elaphus*), African buffalo (*Syncerus caffer*) (29) and moose (*Alces alces*) (8). The organism has also been isolated from rodents, capybara (*Hydrochaeris hydrochaeris*), raccoons (*Procyon lotor*), opossums

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**Fig. 1**

Lesions produced by *Brucella* spp.

Photomicrographs of tissue sections of placentas. Note colonisation of epithelium by brucellae labelled rust-red (1a & 1b) and brown (1c). Sections were stained with immunohistochemical procedures using anti-*B. abortus* antibody.
(Didelphis virginiana) and canids, including coyotes, wolves and foxes (8, 63). Bison, elk and African buffalo are known maintenance hosts of Brucella abortus and may serve as reservoirs of the infection with the potential for transmission to cattle.

In bison, the pathobiology of the disease is considered similar to that in cattle (11, 53, 54). Transmission usually occurs via ingestion of infectious products of parturition. Observational studies have demonstrated the marked interest shown by near-term pregnant bison in parturition events among herd-mates, suggesting an efficient mechanism of transmission of Brucella abortus (unpublished data). Additionally, perinatal infection may occur and persist until adulthood although this does not appear to be common. Most calves born to infected dams have no demonstrable infection and lack Brucella antibody by five months of age, though they may be suckling infected milk (53). Septicaemia occurs early in the course of infection and may be detectable intermittently for at least two years.

In males, infection often localises in the genital organs and associated superficial inguinal and internal iliac lymph nodes (55, 77). Seminal vesiculitis is common, sometimes resulting in palpable nodular enlargements of the seminal vesicles. Histologically, seminal vesicles have lymphoplasmacytic inflammatory cell infiltrates in the interstitium, and colonies of Brucella, macrophages, neutrophils and degenerate cellular debris in vesicle lumina. In some males, severe necrotising and pyogranulomatous orchitis develops and is characterised by numerous zones of caseous necrosis with mineralisation, surrounded by pyogranulomatous and lymphoplasmacytic inflammatory cell infiltrates (5, 55). Large numbers of Brucella organisms can be demonstrated in the necrotic areas and surrounding macrophages. Other affected reproductive organs include the epididymides, ampullae and prostate. Venereal transmission of Brucella abortus in bison has not been demonstrated (58), although intravaginal exposure to the organism can result in seroconversion of females (75) and bulls are known to shed brucellae in the ejaculate (58, unpublished data).

Females often experience third trimester abortion in the pregnancy following infection. Alternatively, they may have full-term, stillborn calves, weak calves that die in the first two weeks of life, or normal calves. Similar to infection in cattle, the organism colonises placental trophoblasts during pregnancy with a resultant placentitis causing abortion and endometritis that persists after abortion or parturition (54, 77). Placentitis is characterised by oedema, focal and diffuse mixed inflammatory cell infiltrates with villus necrosis, and bacterial colonisation of trophoblasts. Other than placentitis, fetal and neonatal lesions are usually mild, with interstitial or broncho-interstitial pneumonia being the most common. Neonates may also have purulent nephritis, local splenic infarction, and lymphocyte depletion of splenic white pulp and lymph node cortices (54). In some cases Brucella abortus can be demonstrated in macrophages in the pulmonary interstitium or in phagocytes in airways. Many bison have successful pregnancies following abortion; however, some experience later abortions following strain 19 infection, as reported by Davis and co-workers (10). It has also been observed that, following abortion, females tend to have less reproductive success than seronegative bison, though the cause has not been determined (23, 53).

Brucella abortus can be isolated from the milk of infected female bison, but in a comparative study of culture-positive tissues in chronically infected cattle and bison, the organism was routinely isolated from mammary gland specimens of cattle but not bison (54). Mammary involvement occurs in bison but perhaps not to the degree it does in cattle. The duration of infection in bison is unknown, is likely to vary among individuals, and should be thought of in years rather than weeks or months. Naturally infected bison with high antibody titres to Brucella abortus tend to retain the high titres for several years, and it has been shown that bison with high titres are usually the animals from which the organism can be successfully cultured (53, 54).

In elk, Brucella abortus infection was first detected by serological testing in 1930 in the National Elk Refuge in Jackson, Wyoming (73), in 1931 in Yellowstone National Park (60), and in Elk Island National Park, Alberta, Canada, in 1957 (5). Brucellosis in elk in the Greater Yellowstone Area is most prevalent in populations that feed on state and federal feedgrounds. Recently, however, it appears to be expanding into populations more remote from feedgrounds. Interestingly, Brucella abortus infection in the conspecific red deer (Cervus elaphus) has been reproduced experimentally (1), but it is not prevalent in countries where exposure to infected cattle probably occurs (8).

The pathogenesis of Brucella abortus infection in elk is considered to be similar to that in cattle and bison (70), though behavioural differences in the species probably account for variations in epidemiology. Lesions observed in experimental infections are not considered dramatic or diagnostic (69). In early infection, lymph nodes are oedematous and contain increased follicles, numerous neutrophils and some eosinophils. In the chronic stages, there is marked sinus histiocytosis and increased numbers of plasma cells in the medullary cords. In males, lesions in the reproductive organs are usually mild and may consist of lymphoplasmacytic prostatitis, seminal vesiculitis, and ampullitis. Mild focal epididymitis and orchitis also occasionally occur.

Brucella abortus infection in female elk often results in abortion of the first calf. In one study, 48% of female elk...
aborted following infection (69). Occasional animals abort subsequent calves. Placental lesions consist of necrosis and mixed inflammatory cell infiltrates in the placentome and intercotyledonary areas. Placental trophoblasts often contain large numbers of brucellae. Intercotyledonary exudate contains desquamated epithelium, macrophages, neutrophils, cellular debris and brucellae. Endometritis, consisting of lymphoplasmacytic infiltrates with occasional giant cells and macrophages in the lamina propria and gland distention with neutrophils and necrotic debris, occurs and may persist for months following infection. Fetal lesions may include interstitial and broncho-pneumonia, lymphadenitis and lymphoid hyperplasia in lymph nodes and spleen, and focal nephritis and hepatitis. Mammary gland involvement is less frequent in elk than in cattle (71) and retained placentas are not commonly observed. Musculoskeletal lesions are commonly seen in elk and consist of hygromata, carpal bursitis, synovitis and tendonitis. Carpal bursitis, synovitis and tendonitis usually occur in chronically infected elk and may result in severe lameness (69). Bacteremia is intermittent, is most frequent early on in infection, and has been detected for up to 68 weeks post infection (PI). The duration of infection in elk is not known but has been recorded at 56 months after initial infection (71).

Natural and experimental infections involving *B. abortus* in moose have been reported (5, 17, 20, 33). Infected moose maintain high numbers of brucellae in many tissues, especially lymph nodes (20). Death may be caused by endotoxic shock. Reported lesions include pericarditis, myocarditis, pleuritis, peritonitis, arthritis, lymphadenitis, orchitis, nephritis, hepatitis and arthritis. Fibrin is often present on serosal, pleural and parietal surfaces of organs. Experimentally, the presence of fibrin and fibrin tags has been related to the duration and severity of infection (20). Antibody responses were detectable within 15 days PI and have been observed to be extremely high in natural and experimental infections. Persistent bacteremia developed in experimentally infected animals. The persistent bacteremia, high numbers of brucellae in tissues, widespread progressive lesions, lack of recovery from infection, and death from infection in moose, suggest that this species is not able to mount an effective cellular immune response to *B. abortus*. The disease is thought to be generally fatal in moose and the species is considered likely to be a dead-end host (20).

Natural *B. abortus* infection of bighorn sheep (*Ovis canadensis*) has been observed in a research facility and was attributable to fence-line contact with aborting infected elk (34). Infection in the sheep resulted in abortion, placenitis, orchitis, epididymitis and lymphadenitis, morphologically consistent with lesions in cattle and bison. The infection also apparently resulted in the death of two rams.

Serological surveys have indicated exposure of many species of wild canids to *Brucella* spp., as summarised by Davis (8). *Brucella abortus* has been isolated from coyotes (*Canis latrans*), wolves (*Canis lupus*) and three species of wild fox. Scanlan and co-workers (62) demonstrated seroconversion and lymph node infection in grey foxes (*Urocyon cinereoargenteus*) up to 49 days following ingestion of *B. abortus*-inoculated dog food. Davis and others (7) isolated *B. abortus* from stomach contents of neonatal coyote pups and post parturient discharge from a naturally infected coyote bitch. Experimental studies showed faecal shedding of *B. abortus* by coyotes following ingestion of the organism and disease transmission to cattle co-mingled with infected coyotes (9). An experimental infection of wolves with *B. abortus* resulted in positive seroconversion by day 10 PI, transient low-grade septicaemia from one to three weeks PI, and sporadic faecal shedding of small numbers of brucellae for up to 50 days PI (68). Neither gross nor microscopic lesions were observed in wolves necropsied 6 and 12 months PI. Based on natural and experimental observations of *B. abortus* infection in wild canids, these species are considered spillover hosts with little role in maintenance of the infection in other populations.

**Brucella suis**

Serological evidence of *Brucella suis* infection was first discovered in feral pigs in Hawaii in 1962 (46) and later confirmed by culture of the organism in South Carolina (78). It is endemic in some feral pig populations in North America and wild boar in Europe (27). Biovars 1 and 3 are present in North America while biovar 2 is present in Europe. The pathogenesis and pathobiology of brucellosis in feral pigs and wild boar are likely to be identical to those in domestic pigs. In North America, *B. suis* infection in feral pig populations is problematic for cattle producers and regulatory officials, since cattle exposure to the agent results in serologically positive cattle. Cattle naturally infected with *B. suis* biovar 1 from feral pigs, and closely monitored for two years, did not experience abortion, did not transmit the agent to co-mingled negative cattle, and did not shed the organism except in the milk, which remained positive for two years (16).

*Brucella suis* biovar 2 also infects European brown hares (*Lepus europaeus*), which serve as a reservoir capable of transmitting infection to wild and domestic pigs. Lesions observed in infected brown hares consist of granulomas, often containing central necrotic areas, in the spleen, lung, liver, kidney and uterus (30).

*Brucella suis* biovar 4 is endemic in Arctic herds of caribou and reindeer (*Rangifer tarandus*). Additionally, it infects muskox (*Ovibos moschatus*) (26), canids (43), moose (32), grizzly bears (*Ursus arctos horribilis*)
Brucella melitensis is serologically indistinguishable from B. abortus or B. suis, which complicates the interpretation of serological surveys in wildlife in some regions. The organism has been isolated from wild species including chamois (Rupicapra rupicapra) (24) and alpine ibex (Capra ibex) (18). Lesions observed in the chamois and ibex included epididymitis and necrotising and granulomatous orchitis in both species, focal necrotising splenitis in the ibex, and nephritis and meningocerebralitis with accompanying brucellae demonstrated by immunohistochemical staining in the chamois.

Marine mammal Brucella species

Brucella spp. were first isolated from marine mammals in 1994 (15, 59) and have since been detected by serology and culture from a wide range of marine species (47, 52). The organisms, now referred to as Brucella pinnipedialis and Brucella ceti (22), are known to produce abortions with placentitis and trophoblast colonisation in bottlenose dolphins (Tursiops truncatus) (40), meningocerebralitis in striped dolphins (Stenella coeruleoalba) (28, 31), and they have been isolated from abscesses and skin lesions from several species (21). Additionally, purulent to granulomatous orchitis with caseous necrosis and calcification has been observed in the testes of two species of baleen whale that were seropositive for brucellosis (48).

Brucella infection of lungworms has been detected in harbour seals (Phoco vitulina) (25) (Fig. 2), a harbour Nile catfish (Clarias gariepinus) experimentally infected with B. melitensis biovar 3 developed persistent detectable antibodies, with organisms isolated from liver and spleen, at 14 days PI (61). A recent survey of 120 Nile catfish captured from Nile canals detected anti-Brucella antibodies in 8.2% and 9.3% of the fish using two different serological tests (14). Brucella melitensis was detected by polymerase chain reaction (PCR) in livers (5%), spleens (5%), kidneys (4.2%) and skin swabs (13.3%) from the fish. Livers and spleens from infected fish were congested, and skin lesions were present on five fish with positive skin swabs. In this region, B. melitensis is endemic in livestock, and animal wastes and carcasses are often dumped in the Nile canals. The presence of B. melitensis on the skin of catfish led investigators to speculate that humans could become infected through puncture wounds, cuts or abrasions while handling fish.
porpoise (*Phocoena phocoena*) (12) and a harp seal (*Phoca groenlandica*) (38). Marine mammal *Brucella* infections are considered potentially zoonotic, having been isolated from a laboratory worker (3), two individuals with neurobrucellosis (intracerebral granulomas) (65), and one patient with spinal osteomyelitis (37).

**Conclusion**

From the few reported experimental and pathological studies of brucellosis in wildlife species, it is apparent that, for the most part, the pathogenesis and pathobiology of the disease are similar to those observed in domestic animals. Several species-specific exceptions exist, however, such as the apparent lethal outcome of *B. abortus* infection in moose and bighorn sheep, the lack of a protective cellular immune response produced by vaccination in elk, and the abortifacient properties of RB51 inoculation in reindeer. Additionally, information on the pathology and epidemiology of brucellosis in marine mammals is almost non-existent. Alternative pathways of transmission such as those involving parasites, or contact with or consumption of fish, should be considered. Further research on brucellosis in wildlife is likely to uncover new information that will increase our understanding of the disease in both wild and domestic species.

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la brucelose chez les animaux sauvages devrait apporter des éclaircissements précieux, avec des applications concrètes dans la gestion de cette maladie aussi bien dans la faune sauvage que chez les espèces domestiques.

Mots-clés

Patogénesis y patobiología de la brucelosis en la fauna salvaje

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Resumen
En poblaciones salvajes se han observado procesos de infección natural por Brucella spp. Dada la similitud general que presentan las lesiones y la evolución de la enfermedad con independencia de la especie de patógeno y anfitrión de que se trate, se considera que la patogénesis de la brucelosis en la fauna salvaje es semejante a la que se ha observado, y estudiado exhaustivamente, en los animales domésticos. Entre dichas similitudes destacan el tropismo por los tejidos reproductivo y mamario, la colonización de trofoblastos por el microorganismo y la observación de patrones histopatológicos parecidos en los órganos, especialmente en el tracto reproductivo. También existen algunas diferencias en la evolución de la enfermedad, atribuibles seguramente a disparidades en la inmunología y la conducta de las distintas especies. Es de prever que profundizando en el estudio de la patogénesis y la patobiología de la brucelosis en la fauna salvaje se pueda adquirir un conocimiento único, aplicable a la lucha contra la enfermedad en especies tanto salvajes como domésticas.

Palabras clave

References


